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Probiotic effect and dietary correlations on faecal microbiota profiles in irritable bowel syndrome

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Objective: Probiotics and nutrient intakes modulate gastrointestinal (GIT) microbiota and symptoms of irritable bowel syndrome (IBS). The extent to which these factors influence the microbiota is relatively unknown. The primary objective of this paper was to investigate the effect of a probiotic on gut microbiota and IBS symptoms. The secondary objective was exploring correlations between dietary intake and gut microbiota.

Design: This study was an extension of a randomised clinical trial (Clinical Trials Registry NCT018867810). Dietary intake was recorded by three-day estimated food records. Faecal samples were collected at three time points: (1) baseline (A), (2) after eight weeks' probiotic supplementation (*Lactobacillus plantarum* 299v) (B) and (3) following a two-week washout period (C). Total *Bacteroides* spp., *Bifidobacteria bifidum* and *Lactobacillus plantarum* were quantified by quantitative real-time polymerase chain reaction (qPCR).

Results: Twenty-eight diarrhoea-predominant IBS (D-IBS) and 24 constipation-predominant IBS (C-IBS) patients participated. *Lactobacillus plantarum* profiles at baseline (A) were significantly different between C-IBS and D-IBS (-0.956 ± 1.239 vs. -1.700 ± 1.239 ; $p = 0.024$). There was no significant change in bacterial counts after completion of the trial (B) and following the washout period (C) between groups. In both groups there were significant direct correlations between fibre and *Lactobacillus plantarum* and inverse correlations between fibre and *Bacteroides* spp. There was no difference in symptom severity scores between treatment and placebo groups during the study.

Conclusion: The probiotic had no effect on symptoms and GIT microbiota. Certain nutrients strongly correlate to certain bacterial profiles, suggesting that nutrients can significantly influence gastrointestinal microbiota composition.

Keywords: diet, gut microbiota, irritable bowel syndrome, probiotic

Introduction

Irritable bowel syndrome (IBS) is the most prevalent functional gastrointestinal (GI) disorder and is estimated to affect one in five people.¹ No specific test exists for the confirmation of IBS and the diagnosis is dependent on the ROME criteria, which are symptom based. Symptoms include abdominal pain or discomfort, irregular bowel movements, flatulence and constipation or diarrhoea. According to the proportion of symptomatic stools, IBS can further be divided into diarrhoea predominant (D-IBS), constipation predominant (C-IBS), mixed (M-IBS) or unclassified (U-IBS).^{2–4} Various pathogenic mechanisms have been proposed for IBS including visceral hypersensitivity, abnormal motor function, low-grade mucosal inflammation, food intolerance and altered GI microbiota, as well as psychosocial and genetic factors.⁵ However, the pathogenesis of IBS remains poorly understood.

Current treatment regimens for IBS are mainly symptom based. From the early days, diet has formed the cornerstone of IBS management, especially high-fibre diets. More recently, multiple randomised controlled trials have shown that the low-fermentable oligo-, di-, mono-saccharides, and polyols (FODMAP) diet is beneficial for the improvement of overall and individual symptoms in IBS.⁶ A number of recent systematic reviews and meta-analyses suggest that probiotics are associated with an improvement in IBS symptoms compared with placebo. However, these results should be interpreted with caution, given the methodological limitations of the contributing studies.^{7–10} Probiotic treatments are routinely recommended in clinical practice to alter

gut microbiota, as dysbiosis has been confirmed in IBS patients.¹¹ The treatment outcomes with these modalities are disappointing, with high failure and recurrence rates and high economic cost.¹² Seemingly, a more structured approach to IBS care is needed.

The human GI microbiota constitutes a complex ecosystem that is beneficial to the host under normal conditions.¹³ GI infection or administration of antibiotics perturbs GI microbiota composition and has been linked to the expression of dysfunctional GI symptoms.¹⁴ Several studies over recent years have demonstrated compositional differences in the intestinal microbiota between IBS and healthy controls.^{15–17} In general, data indicate that the overall microbial diversity of the intestinal microbiota in IBS is reduced compared with the diversity in healthy individuals.^{15,18,19} There is a rationale for targeting the intestinal microbiota in the treatment of IBS.²⁰

The link between diet, microbiota and fermentation products might have an essential role to play in IBS aetiology.¹⁵ Few studies have examined the impact of dietary interventions on the microbiota in IBS patients. A low-FODMAP diet has been linked to reduced *Bifidobacteria* counts,²¹ which seems a paradox given their potential symptom benefit.

There have been relatively few randomised controlled trials (RCTs) that have assessed the effects of a probiotic on IBS symptoms and GI microbiota.²² To an even lesser degree, barring the FODMAP studies, the relationship of nutrient intakes on GI

microbiota is poorly understood in IBS. Whether a disease-prone microbial composition can be transformed into a healthier composition by a probiotic or is influenced by diet to improve patient sense of well-being remains fundamentally an unanswered question. The aim of the present study was to investigate the effect of a probiotic supplement, *L. plantarum* 299v, on the (i) faecal microbiota and (ii) GI symptoms of IBS and, second, to investigate correlations between dietary intake and faecal microbiota.

Materials and methods

Subjects

A total of 52 IBS subjects participated in this study, which formed a part of a larger probiotic RCT (Clinical Trials Registry number NCT01886781) evaluating the efficacy of an eight-week treatment regime of *Lactobacillus plantarum* 299v for IBS.²³ The study was approved by the Health Research Ethics Committee at Stellenbosch University (N10-08-270) and written informed consent to participate was obtained from each participant on enrolment. Twenty-four C-IBS and 28 D-IBS patients were included after screening by a gastroenterologist and recruited according to the study inclusion criteria and their willingness to participate. Detailed methodology followed was discussed in a paper by Stevenson *et al.*²³

Probiotic intervention

The RCT was 12 weeks in duration, with a two-week run-in phase, then active treatment for eight weeks, followed by a two-week washout period. During the intervention, all subjects received either *L. plantarum* 299v or placebo (once daily). Probiotic treatment was given to 19 D-IBS patients and 16 C-IBS patients while 17 patients from both groups received placebo. The test product contained 5×10^9 colony forming units (CFU) of *L. plantarum* 299v and it was tested against placebo capsules, filled with micro-crystalline cellulose powder (mean content of cellulose per capsule 256 mg), of identical taste, texture and appearance by the manufacturer (Ferlot Manufacturing and Packaging (PTY) Ltd, Jeffreys Bay, South Africa). The test product was analysed for viable units and this confirmed packaging quantity details. The dose was two capsules taken orally every morning. Since this was a double-blind study and patients were randomly allocated to receive either the probiotic treatment or placebo, matching for severity of symptoms or for other demographics was not possible.²³ Patient compliance was monitored at six time points during the 12-week trial, with five consultations and one telephonic consult.

Dietary assessment

A registered dietitian explained and trained each participant on the procedure for completing a prospective, three-day estimated (using household food measures) dietary record. The dietary assessment was done only at baseline (A). The importance of food recording immediately after it was eaten was emphasised. The results were analysed by FoodFinder™ III (<https://mrc-foodfinder.software.informer.com/3.0/>), a computer-based data evaluation system for South African foods.²⁴ The reliability of the food records was assessed by means of a test-retest (eight-week interval). Validity was assessed using dietary fatty acid intake from three-day food records and comparing with plasma fatty acid profiles.

Faecal sampling and analysis

Faecal samples were collected at three time points: baseline (A), after eight weeks' supplementation (B) and following a two-

week washout period (C). Samples were collected with disinfected plastic equipment after defecation and immediately frozen, and kept at -20°C for up to a month before being stored at -80°C until analysis. Not all participants provided a stool sample at each time point (A, B and C). Total DNA was extracted using the QIAmp DNA stool extraction mini kit (Qiagen, Hilden GmbH, Germany) with some modifications. The DNA concentration and integrity were determined using a NanoDrop spectrophotometer (NanoDrop Technologies Inc, Wilmington, DE, USA). Only samples with integrity between 1.8 and 2.2 were used.

Total *Bacteroides* spp., *Bifidobacteria bifidum* and total *Lactobacillus plantarum* were quantified using the PrimerDesign™ genesig kits (Primerdesign Ltd, Chandler's Ford, UK) and quantitative real-time polymerase chain reaction (qPCR) amplification and detection. These kits were designed to have the broadest detection profile possible for *in vitro* quantification of all *Bacteroides* species and all *Bifidobacterium bifidum* genomes. A detection kit was specifically developed for *Lactobacillus plantarum* (Primerdesign, UK). Analysis was performed by the Biochemistry and Microbiology Department at Nelson Mandela University.

IBS symptom severity score

The severity of GI symptoms was assessed by a validated questionnaire for use in IBS patients, the Francis Severity Score (FSS).²⁵ The FSS questionnaire was completed at six different time points over the 12-week trial. The questionnaires were self-administered.

Statistical methods

In qPCR analyses, some of the target organisms remained below the detection limit. These values may not have been truly zero or missing values but caused by technical limitations of the qPCR technique. Therefore, for data analysis, the undetected samples were given a value, which corresponded to the limit of detection of the respective qPCR assay. The data were not normally distributed per treatment groups (i.e. placebo vs. probiotic) and per IBS (C-IBS vs. D-IBS) groups. Thus, the variables were transformed with a log transformation to yield more normally distributed data. The analyses showed that the log-transformed data were still not normally distributed. Therefore, the ANOVA comparisons were confirmed with Mann-Whitney U-tests. Correlations among the continuous variables were done with Pearson and Spearman rank correlation coefficients. Repeated measures ANOVAs were done with the assumption of compound symmetry (i.e. equal correlation among the FFS responses over time). Dietary validity data were analysed using Pearson and Spearman correlation coefficients and paired t-tests were used to analyse reliability. The statistical analyses were done with STATISTICA (www.statsoft.com) with a significance level of 5%.

Results

A total of 52 IBS participants were included in this study. Demographic detail and clinical characteristics are shown in Table 1. Participants' mean BMIs fell either into the overweight ($25.0\text{--}29.9\text{ kg/m}^2$) or obese ($> 30\text{ kg/m}^2$) categories. IBS was longstanding i.e. > 5 years for most of the participants. Almost all the participants were female (51 of 52 participants).

At baseline (A), before any probiotic intervention, *Lactobacillus plantarum* profiles were significantly different between C-IBS and D-IBS (-0.956 ± 1.239 vs. -1.700 ± 1.239 ; $p = 0.024$), with lower counts in D-IBS. There was no significant change in

Table 1: Patient demographic and clinical characteristics ($n = 52$)

Factor	D-IBS		C-IBS	
	Treatment ($n = 19$)	Placebo ($n = 9$)	Treatment ($n = 16$)	Placebo ($n = 8$)
Average age (years) (range)	52.2 \pm 16.2 (24.9–75)	42.5 \pm 7.2 (31.9–51.2)	51.5 \pm 9.9 (35.9–69)	49.4 \pm 13.9 (33.0–72)
BMI (kg/m^2) (range)	29.4 \pm 7.4 (19.26–42.0)	33.07 \pm 9.3 (17.19–48.9)	30.9 \pm 7.2 (20.2–49.0)	27.8 \pm 6.3 (21.33–40.0)
Duration of IBS symptoms (years)	7.3 \pm 11.7	7.9 \pm 7.1	12.7 \pm 9.9	12.4 \pm 8.2

No significant differences within (treatment vs. placebo control in D-IBS or C-IBS) or between groups.

D-IBS: diarrhoea-predominant irritable bowel syndrome, C-IBS: constipation-predominant irritable bowel syndrome, BMI: body mass index, IBS: irritable bowel syndrome.

bacterial counts after completion of the trial (B) and following the washout period (C) between groups. Profiles for *Bacteroides* spp. and *Bifidobacteria bifidum* were, however, not different at baseline (A) between the C-IBS and D-IBS groups (data not shown). The probiotic had no significant effect on bacterial profiles between the treatment and placebo groups from baseline (A) to end of treatment (B) in both C-IBS and D-IBS groups (see Table 2). There was no significant change in bacterial counts after completion of the trial (B) and following on into the washout period (C). When the data of only those that provided all three stool samples ($n = 33$) were analysed, no

significant differences were found between the C-IBS and D-IBS groups.

Table 3 gives an overview of the participants' nutrient intake at baseline (A). The C-IBS group had a higher intake of energy and macronutrients, fat, protein and carbohydrate, as well as a slightly higher fibre intake compared with the D-IBS group, although these differences were not significant. A small sub-sample (C-IBS and D-IBS) of the total study group involved in the RCT ($n = 81$) was used to assess validity ($n = 5$, 6.2% respectively) and reliability ($n = 6$, 7.2% respectively) of the dietary

Table 2: Bacterial counts in stool at baseline (A), after supplementation (B) and after washout (C)

Bacteria		D-IBS			C-IBS		
		Mean count \pm SE (log transformed per nanogram DNA)			Mean count \pm SE (log transformed per nanogram DNA)		
		Before (A)	After (B)	Washout (C)	Before (A)	After (B)	Washout (C)
<i>Bacteroides</i>	Treatment group	2.16 \pm 2.49 ($n = 15$)	2.30 \pm 2.85 ($n = 15$)	2.48 \pm 2.91 ($n = 14$)	3.21 \pm 2.61 ($n = 12$)	2.69 \pm 2.60 ($n = 12$)	3.10 \pm 2.52 ($n = 13$)
	Placebo group	1.12 \pm 2.13 ($n = 7$)	2.16 \pm 2.93 ($n = 7$)	1.54 \pm 2.39 ($n = 7$)	2.49 \pm 2.99 ($n = 7$)	2.65 \pm 3.13 ($n = 8$)	2.74 \pm 2.94 ($n = 7$)
<i>Bifidobacteria</i>	Treatment group	-0.69 \pm 1.50 ($n = 15$)	-0.67 \pm 1.67 ($n = 17$)	-0.88 \pm 1.36 ($n = 14$)	-0.16 \pm 1.66 ($n = 13$)	-0.53 \pm 1.44 ($n = 13$)	-0.24 \pm 1.82 ($n = 12$)
	Placebo group	-1.14 \pm 1.14 ($n = 7$)	-1.12 \pm 1.21 ($n = 7$)	-0.95 \pm 1.59 ($n = 7$)	-0.37 \pm 1.66 ($n = 7$)	-0.59 \pm 1.85 ($n = 8$)	-0.46 \pm 1.54 ($n = 6$)
<i>Lactobacillus plantarum</i>	Treatment group	-1.88 \pm 0.00 ($n = 12$)	-1.14 \pm 1.18 ($n = 14$)	-1.34 \pm 0.90 ($n = 13$)	-0.96 \pm 1.31 ($n = 8$)	-0.44 \pm 1.21 ($n = 10$)	-0.89 \pm 1.39 ($n = 12$)
	Placebo group	-1.34 \pm 0.82 ($n = 6$)	-1.80 \pm 0.17 ($n = 6$)	-1.60 \pm 0.58 ($n = 5$)	-0.95 \pm 1.27 ($n = 5$)	-0.96 \pm 1.41 ($n = 7$)	-1.66 \pm 0.48 ($n = 5$)

Mean \pm SE.

No significant differences within (treatment vs. placebo control in D-IBS or C-IBS) or between groups.

D-IBS: diarrhoea-predominant irritable bowel syndrome, C-IBS: constipation-predominant irritable bowel syndrome, SE: standard error, DNA: deoxyribonucleic acid.

Table 3: Nutrient intake of participants ($n = 52$) at baseline (A), mean \pm SD

Factor	All groups	D-IBS ($n = 28$)	C-IBS ($n = 24$)
Energy (MJ)	7.25 \pm 1.95	7.02 \pm 1.76	7.53 \pm 2.22
Total fat (g)	68.30 \pm 22.93	66.13 \pm 20.29	70.84 \pm 26.29
% energy from fat	35.72 \pm 6.78	36.15 \pm 7.35	35.21 \pm 6.05
Total protein (g)	61.04 \pm 17.34	59.35 \pm 17.09	63.02 \pm 18.35
% energy from protein	14.88 \pm 4.57	14.77 \pm 3.91	15.00 \pm 5.14
Total carbohydrate (g)	198.16 \pm 69.29	191.49 \pm 67.30	205.93 \pm 71.13
% energy from carbohydrate	45.83 \pm 7.34	45.64 \pm 7.65	46.05 \pm 7.34
Total dietary fibre (g)	14.14 \pm 7.92	13.66 \pm 7.69	14.72 \pm 8.03
Insoluble dietary fibre (g)	4.07 \pm 2.62	3.99 \pm 2.59	4.16 \pm 2.50
Soluble dietary fibre (g)	3.26 \pm 2.41	3.28 \pm 2.70	3.23 \pm 1.77
Linoleic acid C18:2 (g)	16.11 \pm 7.80	15.91 \pm 7.67	16.34 \pm 8.00
Linolenic acid C18:3 (g)	0.40 \pm 0.19	0.39 \pm 0.17	0.42 \pm 0.20

No significant differences within (treatment vs. placebo control in D-IBS or C-IBS) or between groups.

D-IBS: diarrhoea-predominant irritable bowel syndrome, C-IBS: constipation-predominant irritable bowel syndrome, SD: standard deviation, MJ: megajoule.

data. In the reliability testing, none of the macronutrients differed significantly from each other except that of percentage energy intake from protein 12.33 ± 1.29 vs. 17.48 ± 3.18 g/day ($p = 0.015$). Correlation coefficients for validity ranged from 0.03 to 0.69, $p > 0.05$.

The nutrient intake data from Table 3 were used to correlate to the findings of the faecal microbiota. These data are presented in Table 4 and described in detail by bacterial categories below.

Bacteroides spp.

In the combined C-IBS and D-IBS groups, *Bacteroides* had a significant inverse correlation with total ($r = -0.424$; $p = 0.019$), insoluble ($r = -0.406$; $p = 0.023$) and soluble fibre ($r = -0.466$; $p = 0.008$). In the D-IBS group, *Bacteroides* inversely correlated with total ($r = -0.528$; $p = 0.024$) and soluble dietary fibre ($r = -0.571$; $p = 0.013$). A strong correlation was found in the D-IBS group for percentage energy from fat and *Bacteroides* ($r = 0.617$; $p = 0.006$).

Lactobacillus plantarum

A direct correlation was found for fibre fractions (total [$r = 0.529$; $p = 0.002$], insoluble [$r = 0.465$; $p = 0.008$] and soluble fibre [$r = 0.433$ $p = 0.015$]) and *Lactobacillus plantarum* in the total group. In the D-IBS group a correlation was found for protein intake and *Lactobacillus plantarum* ($r = 0.487$; $p = 0.041$). In the C-IBS group *Lactobacillus plantarum* correlated well with total dietary fibre ($r = 0.584$; $p = 0.036$).

Bifidobacteria bifidum

In the D-IBS group correlations were found for protein intake and *Bifidobacteria* ($r = 0.497$; $p = 0.036$) and *Bifidobacteria* and insoluble fibre ($r = 0.523$; $p = 0.026$) intake. A strong correlation was found in the D-IBS group for linolenic acid (C18:3) intake and *Bifidobacteria* ($r = 0.516$; $p = 0.028$).

There was no significant difference in symptom severity score between the treatment and placebo groups (treatment group 259.54 ± 104.59 – 197.56 ± 114.74 vs. placebo group 258.71 ± 110.88 – 180.00 ± 96.1 ; $p = 0.599$) over the probiotic trial period. The groups were also further divided into C-IBS vs. placebo and D-IBS vs. placebo with no significant differences noted. Both the treatment group and placebo group had a significant improvement in FSS scores over the study period, from an average of 259.27 – 191.71 ($p < 0.0001$) indicating a large placebo effect. A strongly significant positive correlation was found in D-IBS patients receiving placebo at time point B; higher symptom severity score correlated with higher *Lactobacillus plantarum* ($r = 0.892$, $p < 0.05$). A strongly significant inverse correlation was seen in the C-IBS placebo group at time point A; lower *Lactobacillus plantarum* counts translated to a higher symptom severity score ($r = -0.907$, $p < 0.05$). No other significant correlations were found between FSS and microbiota.

Discussion

This study investigated the effects of single strain probiotic supplementation, *L. plantarum* 299v, and nutrient intake correlations on GI microbiota. No significant beneficial effects of the probiotic were observed on severity of IBS symptoms or on GI microbiota composition. However, nutrient intakes were shown to have significant correlations with GI microbiota composition.

Table 4: Correlations between faecal microbiota and nutrient intakes at baseline (A)

Factor	All groups			D-IBS			C-IBS		
	<i>Bacteroides</i>	<i>Bifidobacteria</i>	<i>Lactobacillus plantarum</i>	<i>Bacteroides</i>	<i>Bifidobacteria</i>	<i>Lactobacillus plantarum</i>	<i>Bacteroides</i>	<i>Bifidobacteria</i>	<i>Lactobacillus plantarum</i>
Total fat (g)	0.012	-0.126	0.201	0.207	0.292	-0.161	-0.167	-0.382	0.503
% energy from fat	0.333	-0.087	-0.097	0.617*	0.099	-0.160	0.095	-0.021	0.179
Total protein (g)	-0.235	0.109	0.382*	-0.202	0.497*	0.487*	-0.334	-0.288	0.313
% energy from protein	0.008	0.188	0.031	-0.175	0.106	0.568*	0.127	0.162	-0.289
Total carbohydrate (g)	-0.288	-0.181	0.284	-0.289	0.089	-0.183	-0.312	-0.445	0.551
% energy from carbohydrate	-0.250	-0.161	-0.011	-0.313	-0.126	-0.235	-0.193	-0.245	0.089
Total dietary fibre (g)	-0.424*	-0.057	0.529**	-0.528*	0.175	0.240	-0.443	-0.309	0.584*
Insoluble dietary fibre (g)	-0.406*	0.083	0.465**	-0.417	0.523*	0.253	-0.481	-0.338	0.539
Soluble dietary fibre (g)	-0.466**	0.080	0.433*	-0.571*	0.306	0.319	-0.493	-0.277	0.421
Linoleic acid									
C18:2 (g)	0.056	-0.196	0.126	0.038	-0.784	-0.277	0.081	-0.332	0.407
Linolenic acid									
C18:3 (g)	-0.150	-0.035	0.756	0.218	0.516*	-0.265	-0.503	-0.379	0.279

Significant differences indicated in bold; * $p < 0.05$, ** $p < 0.01$.

D-IBS: diarrhoea-predominant irritable bowel syndrome, C-IBS: constipation-predominant irritable bowel syndrome.

GI microbiota alterations are increasingly being recognised as an important factor in the pathogenesis and pathophysiology of IBS.²⁶ In recent years, many research groups have focused on identifying the GI microbiota composition in IBS patients, using modern culture-independent techniques.²⁷ No single deviance has been identified in IBS microbiota, but various alterations in the bacterial composition have been characterised.^{28–31} In a recent meta-analysis, Hai-Ning *et al.* concluded that there is down-regulation of bacterial colonisation including *Lactobacillus*, *Bifidobacterium* and *F. prausnitzii* in IBS patients, particularly D-IBS.³²

To date there have been very few RCTs on IBS and probiotics that have investigated possible modifications of the microbiota by the probiotic. Knowledge on the role of microbiota modulation in symptom relief is therefore limited.^{22,33} Nobaek *et al.* examined the effect of *L. plantarum* DSM 9843 (299v) on faecal microbiota and IBS symptom relief.³³ There were no significant changes in *Enterobacteriaceae* or sulphite-reducing *Clostridia* or *Enterococci* counts following supplementation, although the *Enterococci* count remained the same in the test group, whereas there was a small increase in the placebo group at the end of supplementation. Flatulence was rapidly and significantly reduced in the test group compared with the placebo group and abdominal pain was reduced in both groups. Kajander *et al.* showed a significant improvement in composite IBS scores with a multispecies probiotic in the treatment group versus placebo group. At the same time they demonstrated a stabilisation of the microbiota: as the microbiota similarity index increased with the probiotic supplementation, it decreased in the placebo group; the difference between the two groups was significant ($p = 0.0015$).²² One recent study comparing the composition and temporal stability of intestinal microbiota between IBS and healthy controls by PCR-DGGE revealed a greater temporal instability in IBS patients (43% instability) than in the control group (29% instability).²⁸ These results suggest that the pathophysiology of IBS may be associated with temporal instability in the composition of intestinal microbiota. However, in our study we found that a probiotic exerted no beneficial changes on the GI microbiota and no consistent correlations were found between GI symptom severity and total *Bacteroides*, *Bifidobacterium bifidum* or *Lactobacillus plantarum* counts. As studies suggest, an association between microbes and symptoms in IBS and the relative importance of different taxa for IBS symptoms has been found to be inconsistent between existing studies.²⁶

Our study has demonstrated strong correlations between certain dietary agents, particularly fibre, and the resulting GI microbiota. It seems as though lower fibre diets predispose towards an increased *Bacteroides* and decreased *Bifidobacterium*, seen in both C-IBS and D-IBS groups. Higher fibre intake was strongly associated with increased *Lactobacillus plantarum* counts in both groups. In the D-IBS group a higher percentage energy from fat and low fibre intake correlated to high *Bacteroides* counts. These findings are in agreement with a previous study by Wu *et al.*³⁴ By combining detailed nutritional analysis and microbiome determination in 98 healthy individuals, Wu *et al.* sought to identify nutrients that substantially affect abundances of microbial species. They found that a higher fat intake and lower fibre intake were associated with the *Bacteroides* enterotype. The nutrient associations seen here parallel a recent study by De Filippo *et al.*³⁵ They compared European children, who eat a typical Western diet high in animal protein and fat, with children in Burkina Faso, who eat

high-carbohydrate diets low in animal protein. The European microbiome was dominated by taxa typical of the *Bacteroides* enterotype, whereas the African microbiome was dominated by the *Prevotella* enterotype.

Recent research has highlighted that dietary intervention aimed at decreasing fermentable carbohydrates and FODMAPs, and as a result an improvement in IBS symptoms, also resulted in the decrease of beneficial *Bifidobacteria*.³⁶ This opens the question as to whether probiotic supplementation is needed in addition to dietary advice to restrict fermentable carbohydrate. Our research has demonstrated that low fibre intake was associated with *Bifidobacteria*. Dietary intake provides an attractive and possibly the easiest therapeutic route to modulate GIT microbiota in IBS. If certain bacterial profiles are ultimately shown to be causally related to disease, then long-term dietary interventions may allow modulation of an individual's bacterial profiles to improve IBS symptoms.³⁴

Strengths of the present study include the simultaneous assessment of microbiota, IBS symptoms and dietary intake. We also divided IBS subjects according to bowel habit sub-type. This study is not without limitations: we quantitatively analysed only a few major groups of bacteria that occur in the faeces and there may have been quantitative shifts between different factions within groups that were not detected in this analysis. The small size of the study population may have failed to detect other significant changes in the microbiota.

Conclusion

Lactobacillus plantarum differs between IBS phenotypes. An eight-week course of the single strain probiotic *L. plantarum* 299v did not result in any significant changes in the GI microflora or GI symptoms. Certain nutrients, especially fibre, strongly correlate to certain bacterial profiles and this may provide an attractive management strategy in IBS treatment.

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